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Morphological variability of *Padaungiella lageniformis* (Arcellinida: Padaungiellidae) from the central part of the Balkan Peninsula

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Summary

The genus *Padaungiella* includes five species characterized by flask-shaped shells covered by predated or collected plates. *Padaungiella lageniformis* is the type species with cosmopolitan distribution. In this paper, morphological variability of *P. lageniformis* based on three populations from the central part of the Balkan Peninsula is presented. A specimen characterized by the pointed shell fundus was observed. Also, several specimens completely covered by square plates were registered; therefore, taxonomic status of *Nebela cordiformis* and *Quadrulella lageniformis* are discussed. In one studied population, shells with very long neck (up to 55 μm) were observed and thus relations of *P. lageniformis* with *P. wetekampi* are discussed. It is concluded that shell width/shell length ratio is a very important taxonomic character for distinguishing *P. lageniformis* (0.45–0.67) and *P. nebeloides* (0.34–0.46). In addition, synonymizing of *P. wailesi* with *P. lageniformis* is proposed.

Key words: biometry, morphometry, protists, taxonomy, testate amoebae

Introduction

The family Padaungiellidae was recently established for three closely related genera characterized by an elongated neck: *Alocodera, Apodera* and *Padaungiella* (Luketa, 2015). These testate amoebae have rigid, flask-shaped shells covered by predated siliceous oval or quadrangular plates. The aperture is terminal and bordered by a thick organic lip. This family comprises nine moss-dwelling species, six of which are poorly studied by morphometric methods: so far, only three studies have been dealing with the morphological variability of padaungiellid testate amoebae (Zapata and Fernández, 2008; Todorov et al., 2010; Luketa, 2015).

Jung (1942a) proposed the genus *Schaudinnia* for *Nebela* species with an elongated neck. As Jung's classification lacks type designations, the name *Schaudinnia* is unavailable. Based on molecular data, Kosakyan et al. (2012) placed members of the genus *Nebela* with an elongated neck into a separate genus named *Padaungiella*. These authors included into the genus *Padaungiella* three very common species (*P. lageniformis, P. tubulata* and *P. wailesi*) and two rare species (*P. nebeloides* and *P. wetekampi*). Taxonomy at the species level within this genus is predominantly based on morphometric data. For example, *P. tubulata, P. wailesi* and *P. lageniformis* have very similar morphology, but shell length is the key character for distinguishing these

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species. In addition, *P. lageniformis* and *P. wetekampi* also have very similar morphology, but neck length is the key character for distinguishing these species.

In the present paper, I report the results of morphometric analysis of testate amoebae from three *P. lageniformis* populations from the central part of the Balkan Peninsula and discuss the taxonomic status of some closely related species.

Material and methods

The material for the present study was collected from three localities in the central part of the Balkan Peninsula (Table 1). Morphological characters and morphometric variables were studied using a light microscope Zeiss Axio Imager A1. Images were captured using an AxioCam MRc5 (Zeiss) digital color camera. Measurements were conducted in the program AxioVision 4.9.1. The following shell parameters were measured: shell length, shell width, aperture width, neck length, neck width (Fig. 1), and area of the optical section (area enclosed by the outline of the shell). The following descriptive statistics were calculated: extreme values (minimum and maximum), median, arithmetic mean, standard error of the arithmetic mean, standard deviation, coefficient of variation (in percentage), skewness and kurtosis. Statistical analysis was conducted using the programs PAST 2.17c and STATISTICA 13.0.

Results

Description of the species

The shell is transparent, colorless or slightly yellow, pyriform, always laterally compressed, rounded aborally, prolonged into a tubular neck. I found one specimen with the pointed shell fundus in *Sphagnum*-dwelling population from the Alagovac Lake region (Fig. 2). The shell is covered by a mixture of predated or collected oval, circular, elongated and quadrangular plates; these plates are usually arranged haphazardly and overlap. Several specimens in population from Šargan Mountain have shells covered mainly by quadrangular plates (Fig. 3). The aperture is oval or like an elongated slit and it is surrounded by a thick organic lip. It is characterized by an uneven and undulating rim. Namely, in frontal view the apertural lip is convex, while in lateral view it is concave.

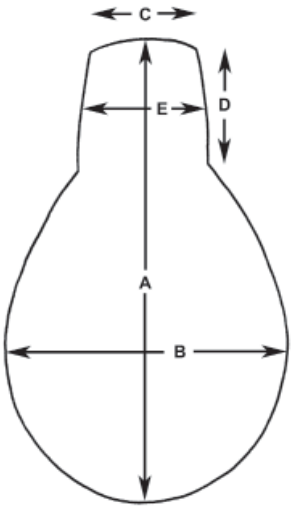

Fig. 1. Shell outline of *Padaungiella lageniformis* and position of the measured axes. A – shell length; B – shell width; C – aperture width; D – neck length; E – neck width.

Population from Šargan Mountain

Morphometric characters of 49 specimens from Šargan Mountain were measured and the results are given in Table 2. Figure 4 shows light micrographs of specimens from this population. The most frequent shell length (131 μm) was registered in 5 specimens (Fig. 5A); the most frequent shell width (72 μm) was registered in 6 specimens (Fig. 5B), and the most frequent aperture width (28 μm) was registered in 14 specimens (Fig. 5C). Coefficient of variation was moderate for area of the optical section (13.75%), while the other measured variables were characterized by low variability (from 4.88% to 9.98%). For basic characters, the minimal variability was observed for aperture width (6.89%), while the maximal variation coefficient was observed for neck length (9.98%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (4.88%), while the maximal variation coefficient was observed for neck width/neck length ratio (9.58%).

Analysis of the size frequency distribution of shell length, shell width and aperture width indicates that this population is size-monomorphic. Shell length ranged from 101 to 153 μm. However, 71.43% of all measured specimens had a shell length of 122–138 μm, whereas only 14.29% were smaller than 122 μm and only 14.29% were larger than 138

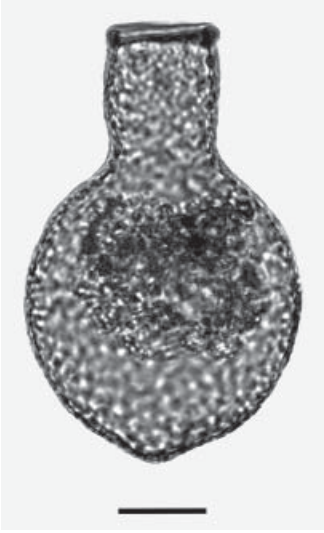

Fig. 2. Specimen of *Padaungiella lageniformis* with pointed fundus of the shell observed in a *Sphagnum*-dwelling population from the Alagovac Lake region, East Herzegovina. Scale bar: 20 µm.

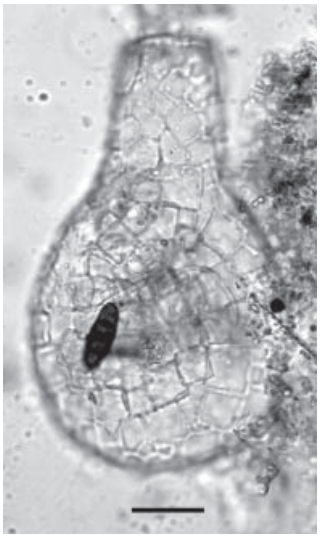

Fig. 3. Specimen of *Padaungiella lageniformis* with shell covered mainly by quadrangular plates observed in a moss-dwelling population from Šargan Mountain. Scale bar: 20 µm.

µm. The frequency analysis of the shell width shows similar distribution pattern. Namely, all measured specimens had shell width ranging between 56 and 90 µm. In this case, 83.67% of all specimens had shell width of 69–80 µm, whereas only 8.16% were narrower than 69 µm and only 8.16% were wider than 80 µm. Figures 5D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

The positive value of skewness for seven characters suggests an asymmetrical distribution with a long tail toward higher values. The asymmetry of aperture width, area of the optical section, neck width/shell length ratio, and neck width/neck length ratio was low, with skewness values ranging between 0.018 and 0.205. Moderate positive values of skewness (0.260–0.381) were observed for shell width/shell length ratio and aperture width/shell width ratio. High positive skewness value (1.200) was observed only for aperture width/shell length ratio. All other variables were characterized by negative values of skewness (between –0.013 and –1.032), indicating a distribution with a long tail toward the lower values.

Two characters (aperture width/shell width ratio and neck length/aperture width ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Since the negative value obtained for neck length/aperture width ratio (–0.215) is not clearly different from zero, the

Table 1. List of sampling locations of the examined populations of *Padaungiella lageniformis* from the central part of the Balkan Peninsula

Microhabitat	Location	Country	Coordinates	Date of collection
epigenous mosses	Šargan Mountain	Serbia	43°49′40.10″N, 19°31′41.40″E	August 17, 2016
epigenous mosses	Alagovac Lake region	Bosnia and Herzegovina	43°17′05.9″N, 18°07′58.7″E	July 24, 2016
Sphagnum mosses	Alagovac Lake region	Bosnia and Herzegovina	43°17′44.8″N, 18°07′31.9″E	April 18, 2014 August 19, 2014 May 11, 2016 July 24, 2016

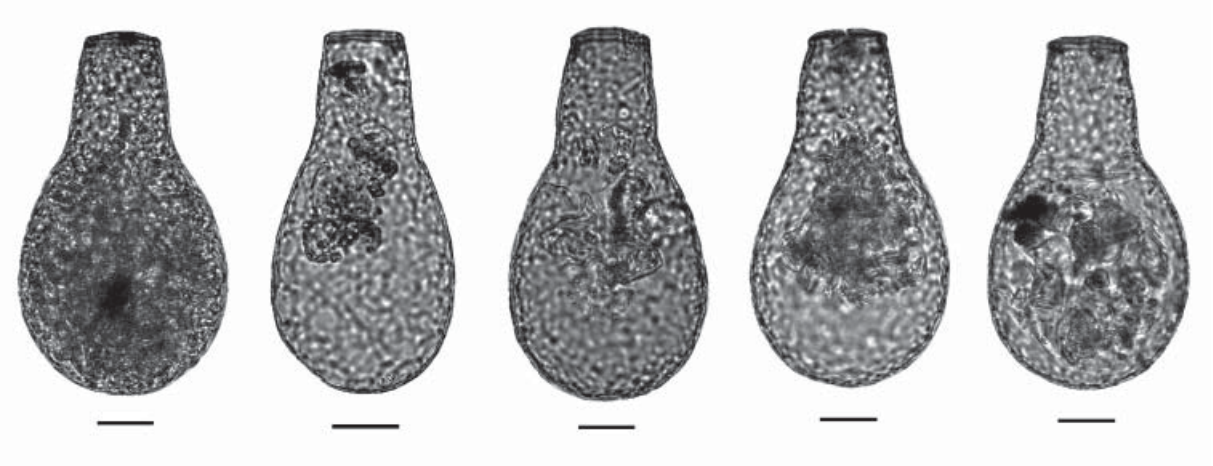

Fig. 4. Light micrographs of *Padaungiella lageniformis*: broad lateral view of different specimens from Šargan Mountain, Serbia. Scale bars: 20 µm.

Table 2. Morphometric characterization of moss-dwelling population of *Padaungiella lageniformis* from Šargan Mountain (Serbia) based on 49 specimens (measurements in µm, except for area of the optical section in µm²)

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	101	153	131	129.67	1.42	9.95	7.67	-0.564	1.323
shell width	56	90	74	73.98	0.82	5.73	7.75	-0.482	2.451
aperture width	23	33	28	28.02	0.28	1.93	6.89	0.205	1.152
neck length	29	48	39	38.47	0.55	3.84	9.98	-0.288	0.290
neck width	26	42	35	35.33	0.43	3.01	8.52	-0.911	1.837
area of the optical section	4270	9684	6792	6796	134	935	13.75	0.018	2.234
shell width/shell length	0.50	0.66	0.57	0.57	0.00	0.03	4.88	0.381	0.943
aperture width/shell length	0.19	0.28	0.21	0.22	0.00	0.02	7.56	1.200	2.729
neck length/shell length	0.24	0.34	0.30	0.30	0.00	0.02	6.44	-0.048	1.112
neck width/shell length	0.20	0.35	0.27	0.27	0.00	0.02	7.99	0.092	4.818
aperture width/shell width	0.34	0.43	0.38	0.38	0.00	0.02	6.32	0.260	-0.776
neck length/shell width	0.43	0.64	0.53	0.52	0.01	0.04	8.17	-0.013	0.203
neck width/shell width	0.37	0.55	0.48	0.48	0.00	0.03	7.20	-0.386	1.060
neck length/aperture width	1.07	1.66	1.36	1.38	0.02	0.13	9.48	-0.045	-0.215
neck width/aperture width	1.00	1.39	1.27	1.26	0.01	0.07	5.83	-1.032	2.171
neck width/neck length	0.65	1.19	0.93	0.92	0.01	0.09	9.58	0.110	2.473

Abbreviations: Min and Max – minimum and maximum values, M – median, x – arithmetic mean, SE – standard error of the arithmetic mean, SD – standard deviation, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

resulting deviation from the normal Gaussian distribution was minimal. However, negative value for aperture width/shell width ratio was clearly different from zero (–0.776), indicating that the average size group has a lower dispersion. Other variables were found to have positive kurtosis values, indicating a distribution that is sharper than the standard Gaussian distribution. Low positive value (0.203) was observed for neck length/shell width ratio, while a moderate positive value (0.290) was observed for neck length. High positive values (0.943–4.818) were observed for all other variables.

Sphagnum-dwelling population from the Alagovac Lake region

Figure 6 shows scatter plot analysis of the correlation between shell length and shell width, indicating presence of two size classes (small-size and large-size) without intermediate specimens. For the specimen with the pointed shell fundus I measured the following values: shell length 102 µm, shell width 64 µm, aperture width 26 µm, neck length 29 µm, neck width 26 µm, and area of the optical section 4281 µm^2. Morphometric characters of 1024 *Sphagnum*-dwelling specimens from the Alagovac Lake region were measured and the results are given in Table 3. Only 15 specimens are included in the small-size class, while the large-size class includes higher number of specimens. Therefore, only the detailed analysis of the large-size class is presented.

Small-size class. Figure 7 shows light micrographs of small-size specimens. Coefficients of variation were moderate for three measured characters (neck length/shell width ratio, neck length/aperture width ratio and neck width/neck length ratio) and ranged from 10.13% to 13.30%; other measured characters had low variability (from 2.82% to 7.76%). For basic characters, the minimal variability was observed for shell length (2.82%), while the maximal variation coefficient was observed for neck length (7.76%). For ratio characters, the minimal variability was observed for neck width/shell width ratio (5.19%), while the maximal variation coefficient was observed for neck length/aperture width ratio (13.30%).

Large-size class. Figure 8 shows light micrographs of large-size specimens. Coefficients of variation were moderate for five measured characters (neck length, area of the optical section, neck length/shell width ratio, neck length/aperture width ratio and neck width/neck length ratio) and ranged from 10.23% to 11.38%; other measured parameters were characterized by low variability (from 4.83% to 8.34%). For basic characters, the minimal variability was observed for shell length (5.49%), while the maximal variation coefficient was observed for area of the optical section (10.95%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (4.83%), while the maximal variation coefficient was observed for neck length/aperture width ratio (11.38%). The most frequent shell length (121 and 123 µm) was registered in 62 specimens (Fig. 9A); the most frequent shell width (69 µm) was registered in 97 specimens (Fig. 9B), and the most frequent aperture width (28 µm) was registered in 239 specimens (Fig. 9C).

Analysis of the size frequency distribution of the measured specimens indicates that this morphometric group is size-monomorphic. For example, shell length ranged from 101 to 139 µm. However, 66.80% of all measured specimens had shell length of 116–128 µm, whereas only 21.01% were smaller than 116 µm and only 12.19% were larger than 128 µm. The frequency analysis of the shell width shows similar distribution pattern. Namely, all measured specimens had shell width ranging between 56 and 83 µm. In this case, 66.11% of all specimens had shell width of 65–73 µm, whereas only 25.67% were narrower than 65 µm and only 8.22% were wider than 73 µm. Figures 9D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

The negative value of skewness (–0.148) for shell length suggests an asymmetrical distribution with a long tail toward lower values. Moderate positive skewness values (0.265–0.468) were observed for neck width/shell length ratio, aperture width/shell width ratio, and neck width/shell width ratio. High positive values (between 0.580 and 1.056) were observed for neck length, aperture width/shell length ratio, neck length/shell length ratio, neck length/shell width ratio, and neck length/aperture width ratio. All other variables were characterized by low positive skewness values (0.084–0.222).

Four characters (shell length, shell width, neck width, and area of the optical section) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Because the negative values obtained for these variables were not clearly different from zero (between –0.002 and –0.187), the resulting deviation from normal Gaussian distribution was minimal. Other variables were found to have positive

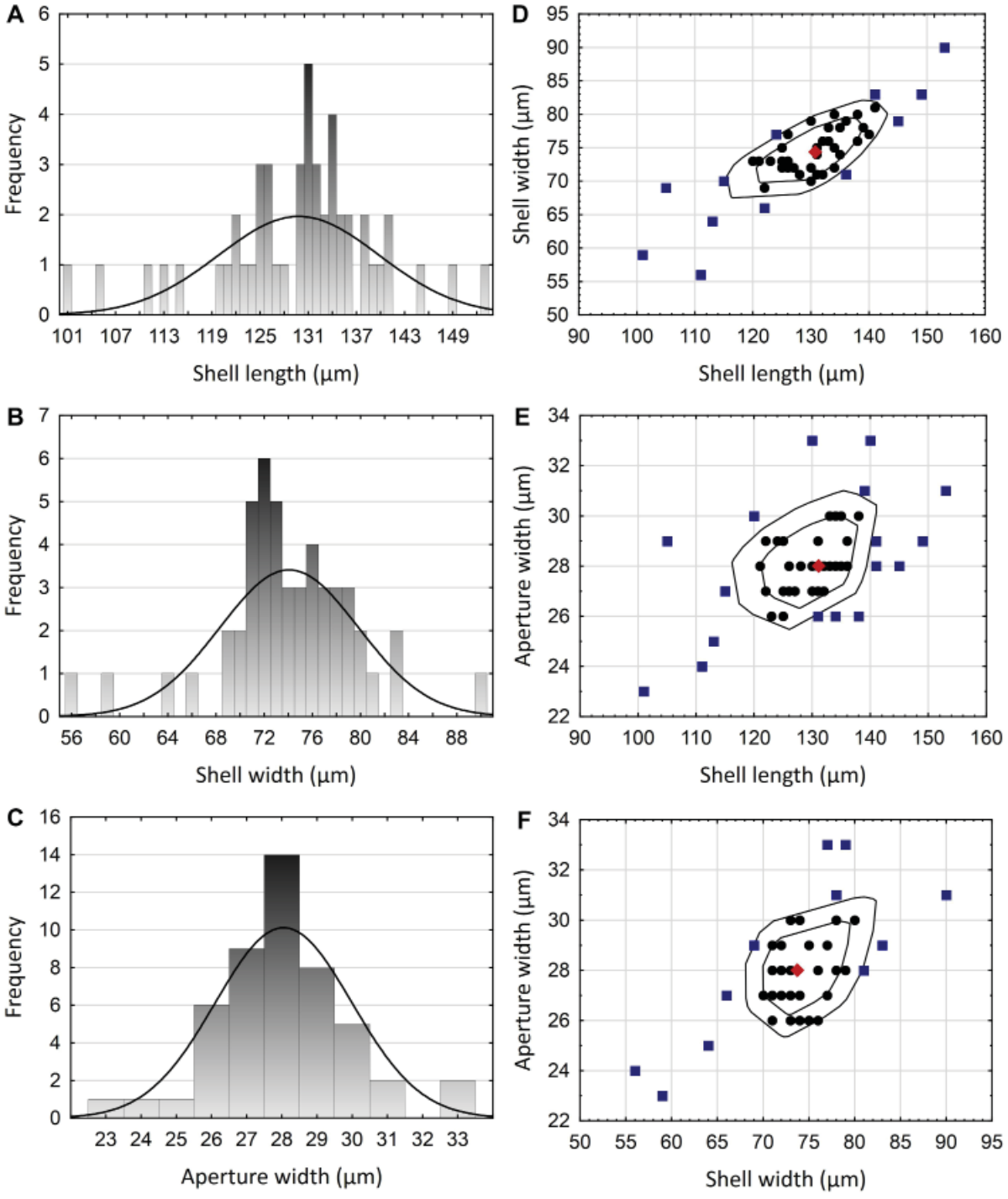

Fig. 5. Morphological variability of *Padaungiella lageniformis* based on 49 specimens from Šargan Mountain (Serbia). Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). Legend for bag plots: depth median ♦, characters on Y axes ●, outliers ■.

Table 3. Morphometric characterization of *Sphagnum*-dwelling population of *Padaungiella lageniformis* from the Alagovac Lake region (East Herzegovina) based on 1024 specimens (small-size class – 15 specimens, and large-size class – 1009 specimens). Measurements are in µm, except for area of the optical section in µm².

Characters	classes	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	small	91	100	97	96.6	0.70	2.72	2.82	—	—
	large	101	139	121	120.99	0.21	6.64	5.49	– 0.148	– 0.187
shell width	small	53	60	55	55.47	0.58	2.26	4.08	—	—
	large	56	83	68	67.50	0.14	4.45	6.59	0.118	– 0.002
aperture width	small	21	27	24	24.27	0.42	1.62	6.69	—	—
	large	23	35	28	27.69	0.05	1.71	6.16	0.220	0.290
neck length	small	22	30	27	26.6	0.53	2.06	7.76	—	—
	large	23	55	34	34.12	0.11	3.58	10.48	0.911	2.685
neck width	small	24	29	26	26.7	0.37	1.45	5.43	—	—
	large	25	38	31	31.16	0.07	2.16	6.95	0.222	– 0.042
area of the optical section	small	3450	4245	3783	3793	46.85	181	4.78	—	—
	large	4010	7798	5717	5718	19.72	626	10.95	0.125	– 0.150
shell width/shell length	small	0.54	0.63	0.57	0.57	0.01	0.03	5.35	—	—
	large	0.45	0.67	0.56	0.56	0.00	0.03	4.83	0.139	0.676
aperture width/shell length	small	0.21	0.28	0.25	0.25	0.00	0.02	7.51	—	—
	large	0.18	0.30	0.23	0.23	0.00	0.01	6.13	0.580	1.622
neck length/shell length	small	0.23	0.31	0.27	0.28	0.01	0.02	7.46	—	—
	large	0.21	0.40	0.28	0.28	0.00	0.02	8.34	0.670	1.707
neck width/shell length	small	0.24	0.30	0.28	0.28	0.00	0.02	6.60	—	—
	large	0.20	0.32	0.26	0.26	0.00	0.01	5.79	0.411	1.114
aperture width/shell width	small	0.38	0.49	0.43	0.44	0.01	0.03	5.86	—	—
	large	0.35	0.51	0.41	0.41	0.00	0.02	5.70	0.265	0.220
neck length/shell width	small	0.36	0.56	0.49	0.48	0.01	0.05	10.13	—	—
	large	0.35	0.80	0.50	0.51	0.00	0.05	10.78	1.056	3.146
neck width/shell width	small	0.43	0.53	0.48	0.48	0.01	0.02	5.19	—	—
	large	0.39	0.57	0.46	0.46	0.00	0.02	5.15	0.468	0.855
neck length/ aperture width	small	0.81	1.43	1.08	1.10	0.04	0.15	13.30	—	—
	large	0.79	2.12	1.22	1.24	0.00	0.14	11.38	1.043	3.879

Table 3. Continuation.

Characters	classes	Min	Max	M	x	SE	SD	CV	Sk	Ku
neck width/aperture width	small	0.96	1.19	1.12	1.10	0.02	0.06	5.29	—	—
	large	0.87	1.32	1.12	1.13	0.00	0.06	5.62	0.084	0.083
neck width/neck length	small	0.83	1.32	1.00	1.01	0.03	0.12	12.33	—	—
	large	0.54	1.38	0.91	0.92	0.00	0.09	10.23	0.125	1.726

Abbreviations: Min and Max – minimum and maximum values, M – median, x – arithmetic mean, SE – standard error of the arithmetic mean, SD – standard deviation, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

kurtosis values, indicating a distribution which is sharper than a standard Gaussian distribution. Low positive values were observed for neck width/aperture width (0.083) and aperture width/shell width (0.220). A moderate positive value (0.290) was observed for aperture width. All other parameters were characterized by high positive skewness values (0.676–3.879).

Moss-dwelling population from the Alagovac Lake region

Figure 10 shows scatter plot analysis of the correlation between shell length and shell width, indicating that this population is size-monomorphic. Morphological variability of this population is illustrated in Figure 11. Morphometric characters of 403 specimens were measured and the results are given in Table 4. Coefficients of variation were moderate for four measured characters (area of the optical section, neck length/shell width ratio, neck length/aperture width ratio and neck width/neck length ratio) and ranged from 10.33% to 12.72%; other measured characters were described by low variability (from 5.23% to 9.54%). For basic characters, the minimal variability was observed for shell length (5.83%), while the maximal variation coefficient was observed for area of the optical section (12.72%). For ratio characters, the minimal variability was observed for shell width/shell length ratio (5.23%), while the maximal variation coefficient was observed for neck length/aperture width ratio (11.20%). The most frequent shell length (110 µm) was registered in 32 specimens (Fig. 12A); the most frequent shell width (58 µm) was registered in 39 specimens (Fig. 12B), and the most frequent aperture width (24 µm) was registered in 101 specimen (Fig. 12C).

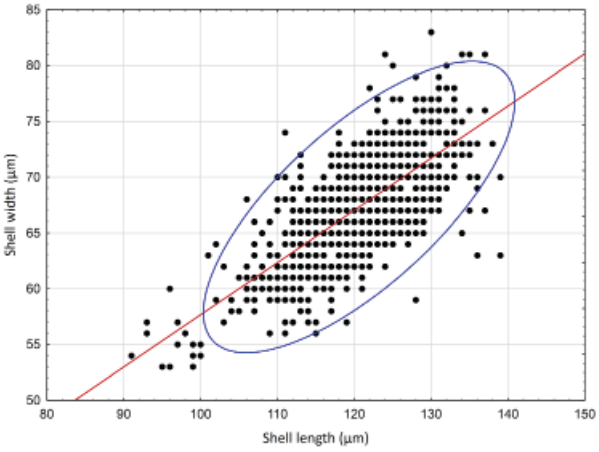

Fig. 6. Scatter plot shows the correlation between shell length and shell width of 1024 specimens of *Padaungiella lageniformis* from a *Sphagnum*-dwelling population near Alagovac Lake, East Herzegovina. Ellipse represents 98% confidence interval.

Analysis of the size frequency distribution of shell length, shell width and aperture width indicates that this population is size-monomorphic. Shell length ranged from 93 to 128 µm. However, 64.27% of all measured specimens had shell length of 105–115, whereas only 19.60% were smaller than 105 µm and only 16.13% were larger than 115 µm. The frequency analysis of the shell width shows similar distribution pattern. Namely, all measured specimens had shell width ranging between 47 and 73 µm. In this case, 68.24% of all specimens had shell width of 55–63 µm, whereas only 13.65% were narrower than 55 µm and only 18.11% were wider than 63 µm. Figures 12D–F show bag plots analyses of the correlation between shell length, shell width and aperture width.

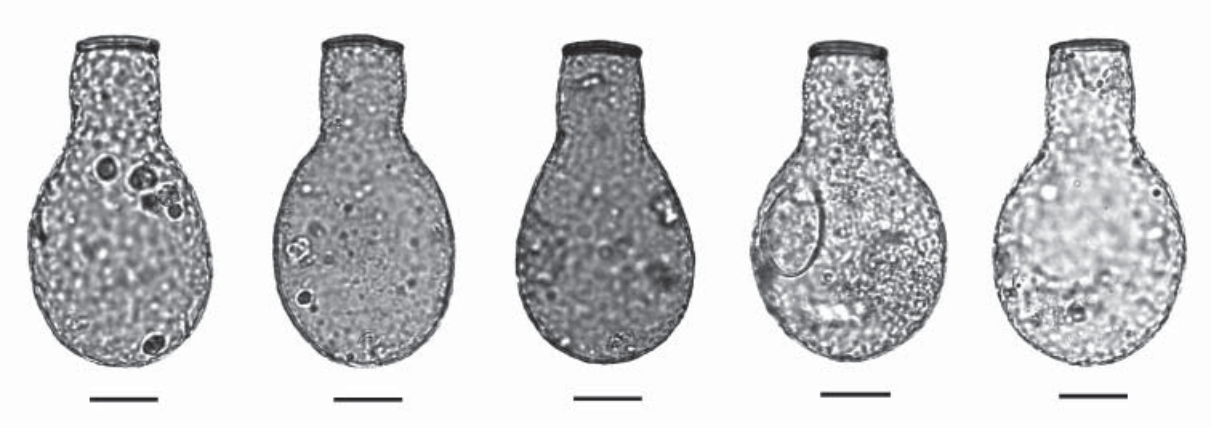

Fig. 7. Light micrographs of small specimens of *Padaungiella lageniformis* from a *Sphagnum*-dwelling population near Alagovac Lake (East Herzegovina) – broad lateral view of different specimens showing general shell shape and outline. Scale bars: 20 µm.

Table 4. Morphometric characterization of moss-dwelling population of *Padaungiella lageniformis* from the Alagovac Lake region (East Herzegovina) based on 403 specimens (measurements in µm, except for area of the optical section in µm²)

Characters	Min	Max	M	x	SE	SD	CV	Sk	Ku
shell length	93	128	110	109.58	0.32	6.38	5.83	0.132	0.002
shell width	47	73	58	59.21	0.24	4.73	7.98	0.537	–0.032
aperture width	21	34	25	25.10	0.09	1.76	7.02	0.811	1.985
neck length	23	42	32	32.09	0.15	3.06	9.54	0.253	–0.122
neck width	24	38	28	28.39	0.10	2.10	7.40	0.668	1.207
area of the optical section	3214	6592	4542	4594	29.12	585	12.72	0.693	0.533
shell width/shell length	0.45	0.63	0.54	0.54	0.00	0.03	5.23	0.295	0.002
aperture width/shell length	0.20	0.32	0.23	0.23	0.00	0.01	6.24	0.972	4.325
neck length/shell length	0.24	0.37	0.29	0.29	0.00	0.02	8.30	0.229	–0.172
neck width/shell length	0.22	0.33	0.26	0.26	0.00	0.02	5.99	0.639	1.290
aperture width/shell width	0.34	0.58	0.42	0.43	0.00	0.03	6.49	0.580	1.937
neck length/shell width	0.41	0.71	0.54	0.54	0.00	0.06	10.46	0.232	–0.177
neck width/shell width	0.41	0.58	0.48	0.48	0.00	0.03	6.27	0.522	0.492
neck length/ aperture width	0.85	1.68	1.27	1.28	0.01	0.144	11.20	0.349	0.124
neck width/aperture width	0.91	1.36	1.13	1.13	0.00	0.06	5.69	0.170	0.946
neck width/neck length	0.67	1.19	0.90	0.89	0.00	0.09	10.33	0.199	–0.204

Abbreviations: Min and Max – minimum and maximum values, M – median, x – arithmetic mean, SE – standard error of the arithmetic mean, SD – standard deviation, CV – coefficient of variation in %, Sk – skewness, Ku – kurtosis.

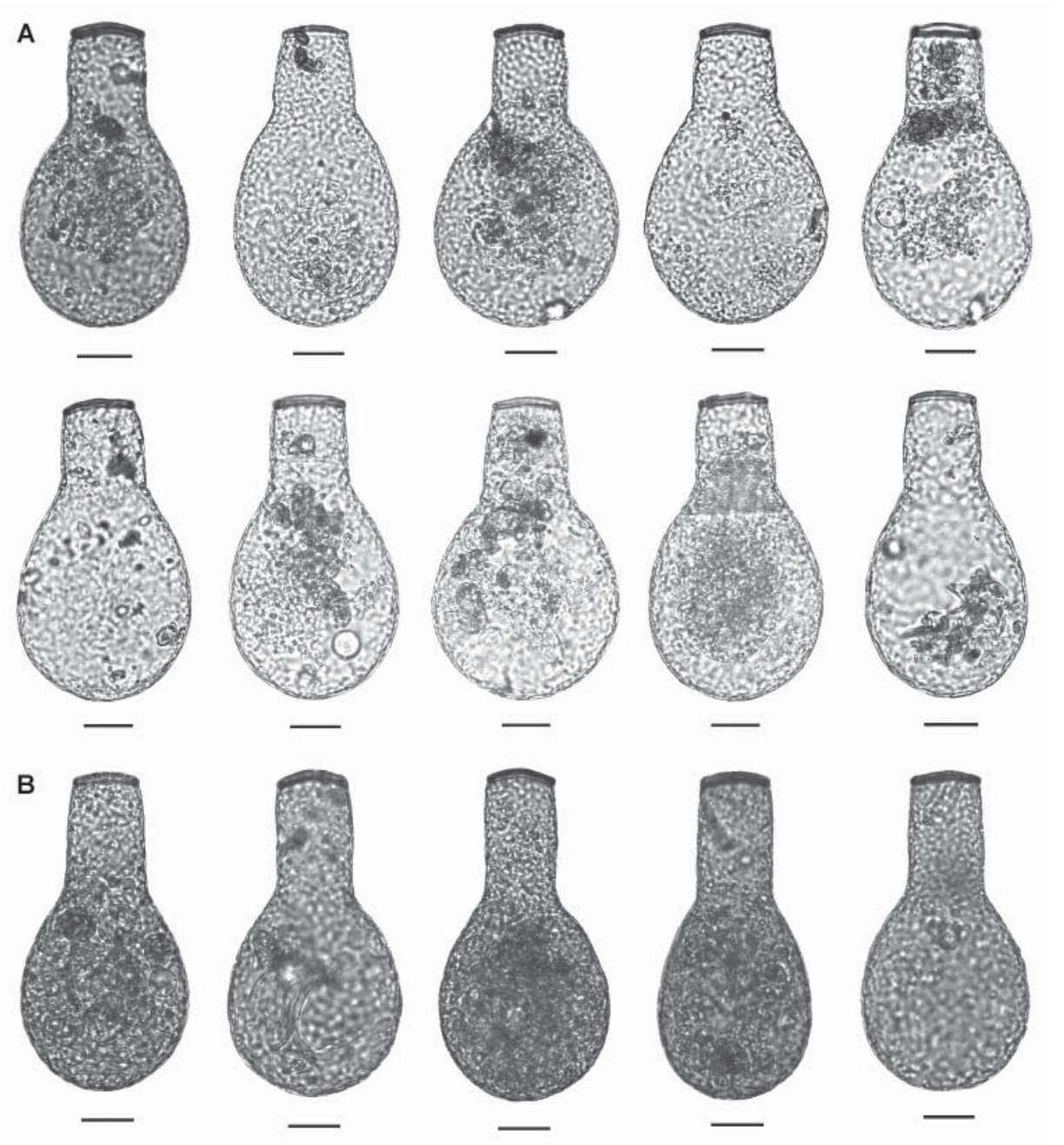

Fig. 8. Light micrographs of large specimens of *Padaungiella lageniformis* from a *Sphagnum*-dwelling population near Alagovac Lake (East Herzegovina) – broad lateral view of different specimens showing general shell shape and outline. A – typical specimens; B – specimens with elongated neck. Scale bars: 20 μm.

The negative values of skewness were not observed for any characters. Namely, all measured variables were characterized by an asymmetric distribution with a long tail toward higher values. The asymmetry of shell length, neck length/shell length ratio, neck length/shell width ratio, neck width/aperture width ratio, and neck width/neck length ratio was low, with a skewness values between 0.132 and 0.232. Moderate positive skewness (0.253–0.349) was observed for neck length, shell width/shell length ratio, and neck length/aperture width ratio. All other variables were characterized by high positive skewness (between 0.522 and 0.972).

Five characters (shell width, neck length, neck

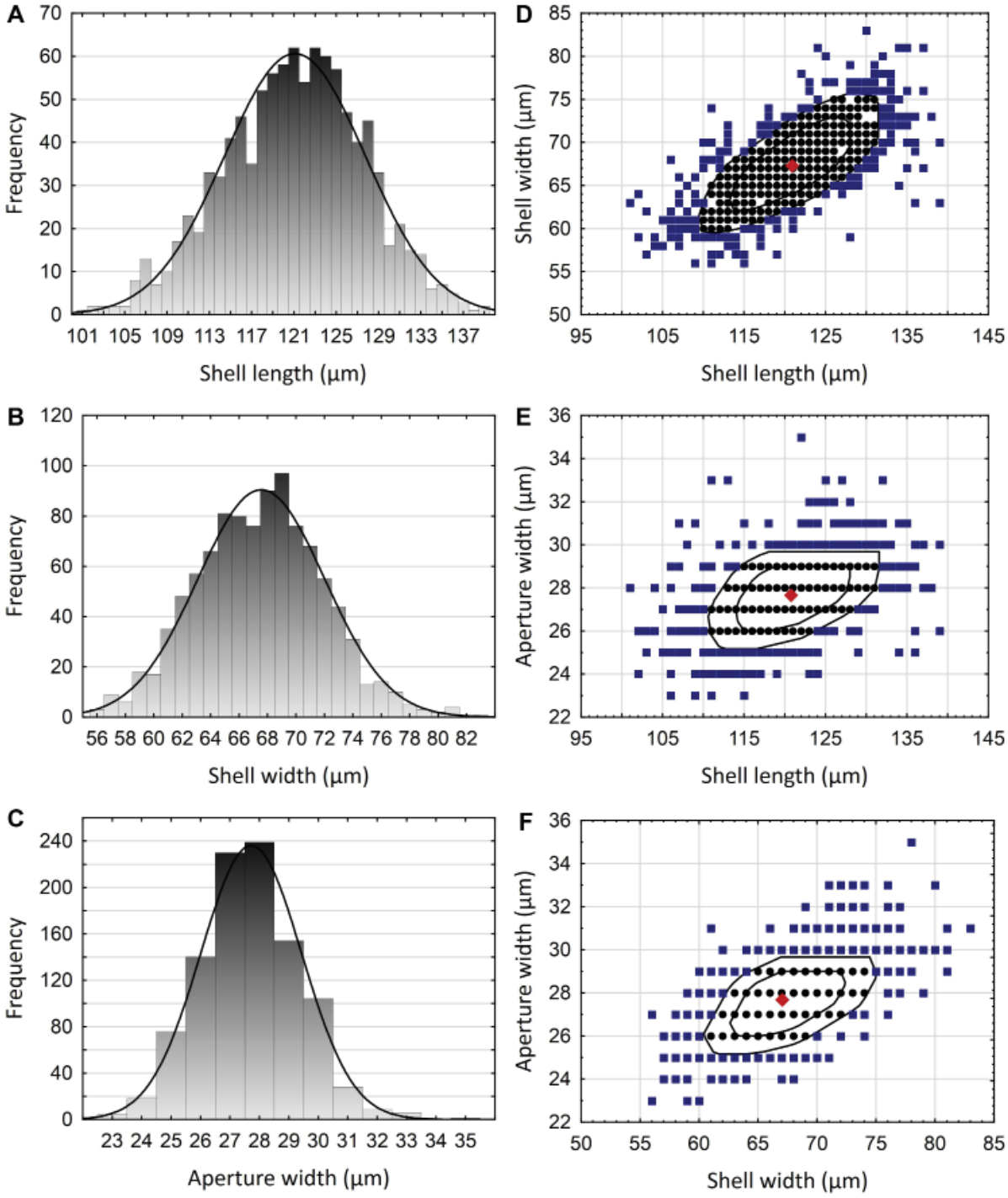

Fig. 9. Morphological variability of 1009 *Sphagnum*-dwelling large specimens of *Padaungiella lageniformis* from the Alagovac Lake region (East Herzegovina). Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). Legend for bag plots: depth median ♦, characters on Y axes ●, outliers ■.

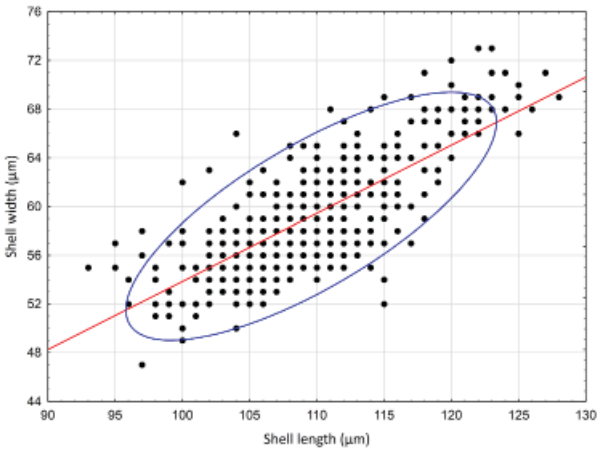

Fig. 10. Scatter plot shows the correlation between shell length and shell width of 403 specimens of *Padaungiella lageniformis* from a moss-dwelling population near Alagovac Lake, East Herzegovina. Ellipse represents 90% confidence interval.

length/shell length ratio, neck length/shell width ratio, and neck width/neck length ratio) displayed negative kurtosis values, meaning that they were characterized by flatter distribution than a standard Gaussian distribution. Because the negative values obtained for these characters were not clearly different from zero (between –0.032 and –0.204), the resulting deviation from normal Gaussian distribution was minimal. Other variables were found to have positive kurtosis values, indicating a distribution which is sharper than a standard Gaussian distribution. Low positive values (between 0.002 and 0.124) were observed for shell length, shell width/shell length ratio, and neck length/aperture width ratio. A moderate positive value (0.492) was observed for neck width/shell width ratio. All other variables were characterized by high positive kurtosis values (between 0.533 and 4.325).

Discussion

There are a number of *Quadrulella* species that differ from the tear-shaped morphology of *Q. symmetrica* complex. These taxa are characterized by shell shapes that are similar to some species from the other genera: *Q. vas* and *Q. constricta* (as *Apodera vas*), *Q. lageniformis* (as *Padaungiella lageniformis*), and *Q. tubulata* (as *P. tubulata*). Kosakyan et al. (2016) noted: "These 'mirror' species could either be a result of convergent evolution or represent cases of the 'classical' hyalosphenids (*A. vas, Padaungiella* ssp.) that live in environments where euglyphids are rare but *Quadrulella* are abundant enough to provide material for building their shells. We suggest that at this point these *Quadrulella* species must be treated as incertae sedis, and their sequencing will certainly illuminate the conundrum of the evolution of square-shaped plates" (Kosakyan et al., 2016, p. 617).

In the population of *P. lageniformis* from Šargan Mountain (Serbia) analyzed in the present study I found several specimens completely covered by square plates of *Quadrulella* members. Namely, this population lives in the environment where euglyphids are rare but *Q. symmetrica* s.l. and *Q. longicollis* are abundant. I think that *Q. lageniformis* and *P. lageniformis* are different species with square plates originated by different ways – *Q. lageniformis* possess self-secreted plates, while *P. lageniformis* possess predated or collected plates. Van Oye (1949) reported clearly different morphometric data and shape of shell plates for these two species from the same locality. Namely, he noted smaller dimensions for *P. lageniformis* (shell length 107–156 µm, shell width 62–80 µm) than for *Q. lageniformis* (shell length 170–192 µm, shell width 85–115 µm). If *Q. lageniformis* possesses predated or collected square shell plates, why then the typical *P. lageniformis* from the same environment do not have square plates? However, without the detailed observations of *Q. lageniformis* based on material from the type population (or another population from Indonesia) all explanations are speculative.

Taxonomy at the species level within the genus *Padaungiella* is predominantly based on morphometric data. Comparative morphometric data of *P. lageniformis* and closely related taxa according to different authors are presented in Table 5.

Heinis (1914) described *Nebela lageniformis* var. *cordiformis* from *Sphagnum* mosses collected in the Páramo de Cruz Verde, eastern branch of the Colombian Andes (South America). Jung (1942b) concluded that this taxon is a separate species and made a new taxonomic combination: *Nebela cordiformis*. This taxon is closely related to *P. lageniformis* but possesses the pointed end of the shell. Heinis (1914) noted only values for shell length: 128–135 µm. Van Oye (1949) reported two specimens from Indonesia and noted similar values for shell length (132–140 µm). Because this

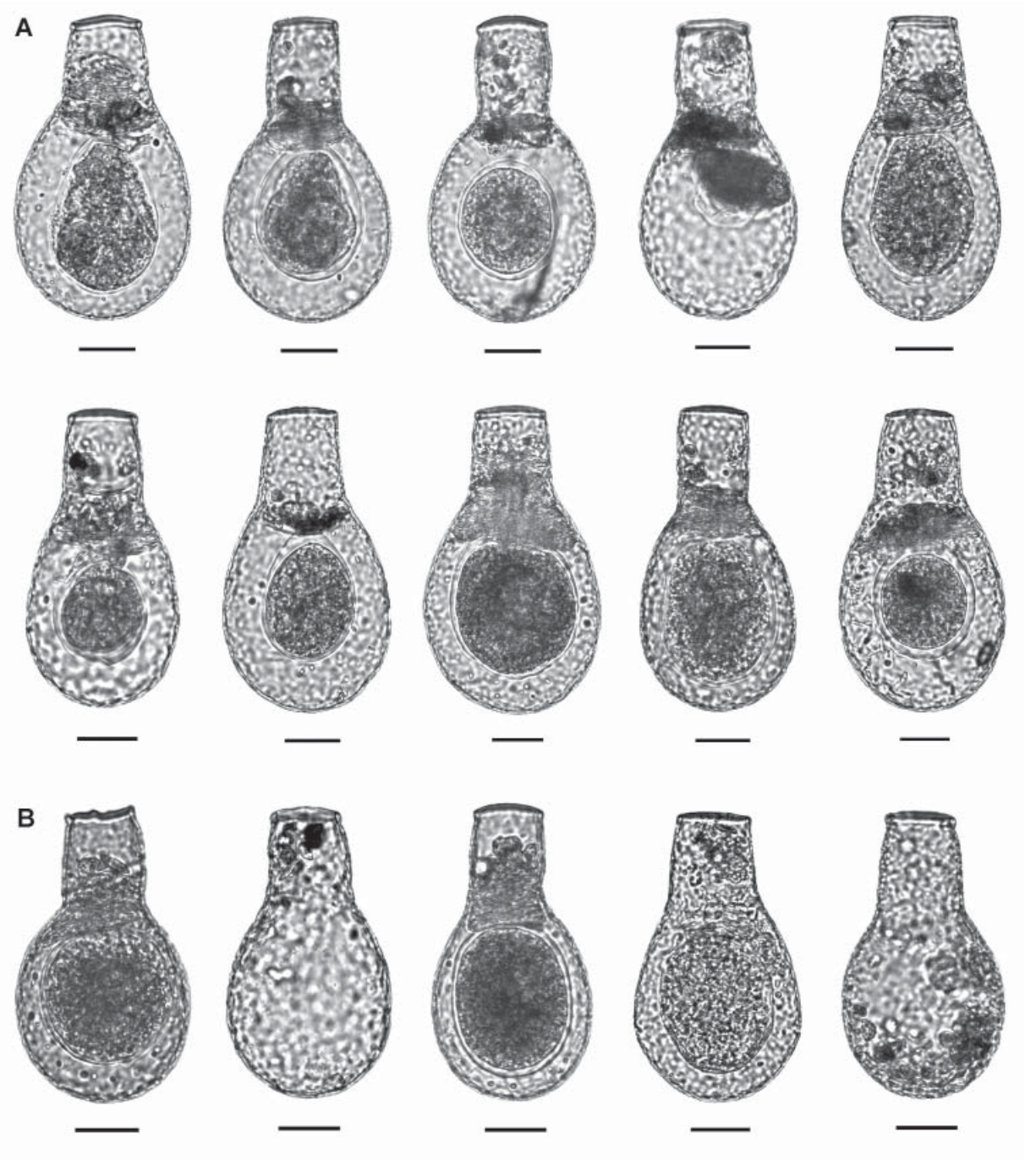

Fig. 11. Light micrographs of *Padaungiella lageniformis* specimens from a moss-dwelling population near Alagovac Lake (East Herzegovina) – broad lateral view of different specimens showing general shell shape and outline. A – typical specimens; B – specimens long up to 100 µm. Scale bars: 20 µm.

taxon is not abundant in any population, detailed morphometric data are not available. Kosakyan et al. (2012) did not mention this species in the list of *Padaungiella* members. The specimen with the pointed shell fundus observed in the present study is very similar to the specimens described from Colombia, but have smaller shell length. I think that it is a mutant specimen because I have found only one specimen with the pointed shell fundus. Unfortunately, Heinis (1914) did not report the number of the observed specimens. Further molecular and morphometric studies are needed to

Table 5. Comparative morphometric data (minimum–maximum) of *Padaungiella lageniformis* and closely related taxa according to different authors (all measurements in μm)

Species	Shell length	Shell width	Aperture width	Neck length	References
P. lageniformis	125–130	70–82	28–32	—	Penard, 1890
	100–153	53–93	—	33–40	Hoogenraad and de Groot, 1940
	103–113	50–73	—	33–50	Hoogenraad and de Groot, 1940
	110–130	57–70	—	—	Hoogenraad and de Groot, 1940
	103–123	51–80	—	—	Hoogenraad and de Groot, 1940
	107–143	53–70	—	—	Hoogenraad and de Groot, 1940
	117–130	60–70	—	40–50	Hoogenraad and de Groot, 1940
	119–131	68–85	28–32	40	Jung, 1942a, 1942b
	107–156	62–80	25–39	30–46	van Oye, 1949
	160	85	35	50	Gauthier-Lièvre, 1953
	98–120	50–69	19–25	—	Ogden, 1984
	125–152	62–78	21–23	—	Chung and Choi, 1995
	125–138	63–75	23–28	53–59	Chattopadhyay and Das, 2003
	91–127	51–73	21–30	23–39	Luketa, 2015
	101–153	56–90	23–33	29–48	This study
	91–139	53–83	21–35	22–55	This study
	93–128	47–73	21–34	23–42	This study
P. nebeloides	118–140	35–45	17–20	—	Gauthier-Lièvre and Thomas, 1958
	113–126	45–53	19–23	35–45	Meisterfeld, 1979
	113–140	43–54	17–21	33–45	Todorov et al., 2010
	115–125	44–54	18–20	33–38	Todorov et al., 2010
P. wailesi	75–100	50–55	25–30	—	Deflandre, 1936
	91–105	50–58	22–28	30–34	Jung, 1942a, 1942b
	75–85	53–58	17–22	25–31	Chattopadhyay and Das, 2003
P. wetekampi	149–159	60–70	26–33	63–68	Jung, 1942a, 1942b
Nebela cordiformis	128–135	—	—	—	Heinis, 1914
	132–140	87–95	—	43–50	van Oye, 1949

clarify taxonomic status of this taxon.

Jung (1942a) described *Schaudinnia wetekamp*i as a pyriform species with a very long pronounced neck. He noted the following measurements: shell length 149–159 μm, shell width 60–70 μm, shell depth 30–47 μm, aperture width 26–33 μm, neck length 63–68 μm, and neck width 25 μm. Jung (1942a) believed that Playfair (1917) observed *S. wetekampi* instead of *Nebela militaris* var. *tubulata*. However, the specimens described by Playfair (1917) have smaller dimentions: shell length 60–64 μm, shell width 26–30 μm, and aperture width 9–14 μm. Kosakyan et al. (2012) included *S. wetekampi* in the list of *Padaungiella* members because it is very similar to the type species – *P. lageniformis*. Namely, key difference between these two species is length of neck. Chattopadhyay and Das (2003) described specimens of *P. lageniformis* with very long neck (53–59 μm) based on material from India. In the present study, I also observed specimens with long neck (up to 55 μm). However, intermediate forms between shells with short neck and shells with long neck were also observed. Unfortunately, detailed morphometric data for *P. wetekampi* are

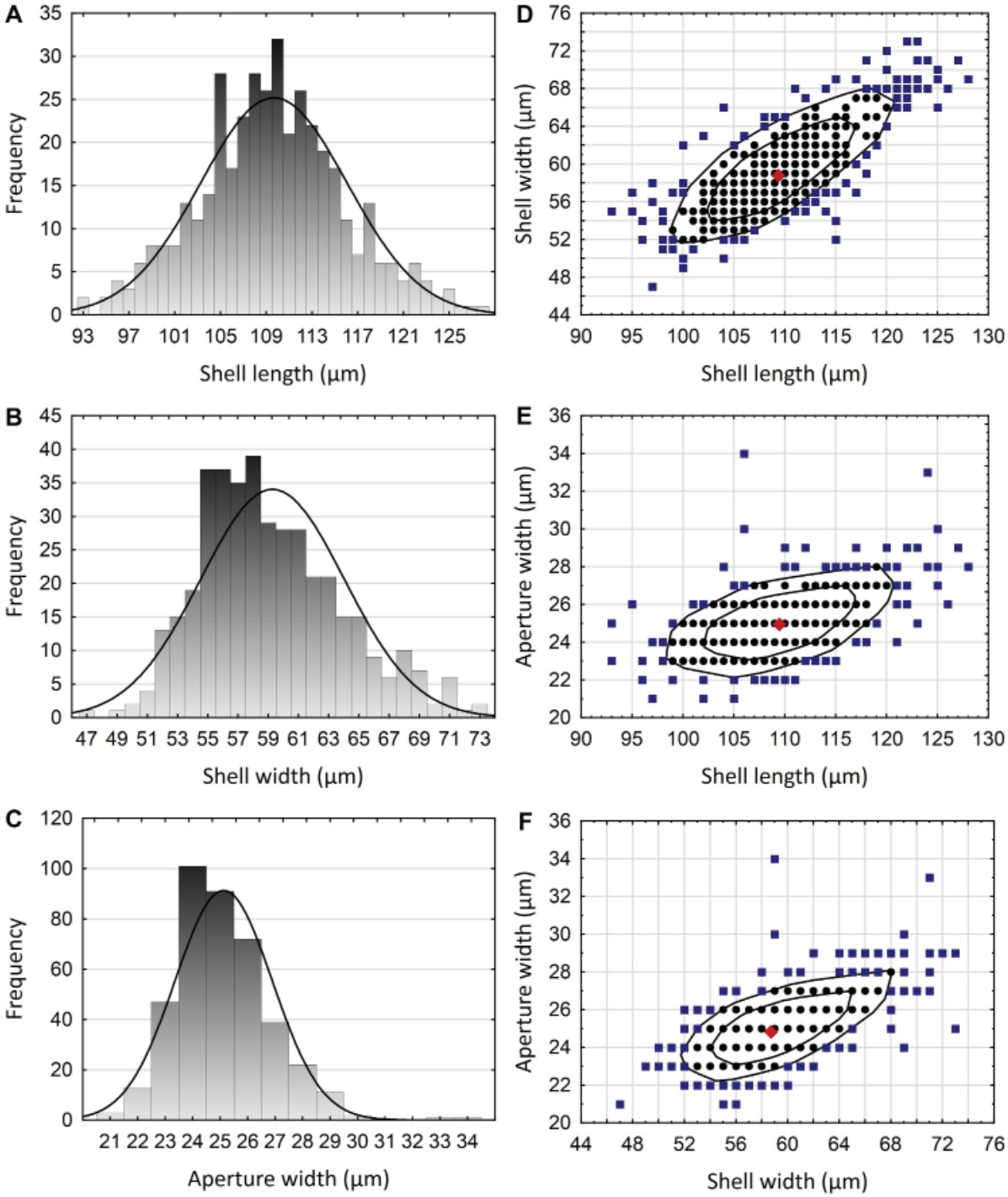

Fig. 12. Morphological variability of *Padaungiella lageniformis* specimens from moss-dwelling population near Alagovac Lake (East Herzegovina) based on 403 specimens. Histograms show the size frequency distribution of the shell length (A), shell width (B), and aperture width (C); bag plots show the correlation between shell length and shell width (D), aperture width and shell length (E), and aperture width and shell width (F). Legend for bag plots: depth median ♦, characters on Y axes ●, outliers ■.

not available. Further studies on morphological variability of *P. wetekampi* are needed to clarify taxonomic status of this species.

Gauthier-Lièvre and Thomas (1958) described *Difflugia nebeloides* from Africa. Todorov et al. (2010) based on two populations from Bulgaria and Austria reported morphological, morphometrical and ecological characteristics of *D. nebeloides* and included this species into the genus *Nebela*. However, Lara and Todorov (in Kosakyan et al., 2012) based on molecular data included this species into the genus *Padaungiella*. The shell of *P. nebeloides* is circular in transverse section, while *P. lageniformis* have laterally compressed shell. Todorov et al. (2010) noted that values of shell width/shell length ratio ranged between 0.34 and 0.46. Van Oye (1949) reported seven specimens of *P. lageniformis* characterized by shell width/shell length ratio values between 0.51 and 0.58. Luketa (2015) calculated values of shell width/shell length ratio for *P. lageniformis* based on specimens from the Vlasina Lake region (Serbia) and noted that values of this ratio ranged from 0.46 to 0.63. In addition, all specimens of *P. lageniformis* analyzed in the present study have similar ratio of shell width and shell length (0.45–0.67). It is possible to conclude that shell width/shell length ratio is a very important taxonomic character for distinguishing these two species. Unfortunately, Todorov et al. (2010) did not calculate ratio values for the other characters.

Polymorphism is simultaneous presence of two or more phenotypes in a population or interrupted diversity of forms on the common genetic base. There are two types of this phenomenon: continuous and discontinuous polymorphism. In the cases of continuous polymorphism, it is not possible to distinguish clearly defined size classes. This type of polymorphism is relatively common among testate amoebae (Schönborn and Peschke, 1988; Schönborn, 1992; Golemansky and Todorov, 2006; Todorov, 2010). However, so far size discontinuous polymorphism was registered only in three cases among testate amoebae: *Centropyxis plagiostoma* (Foissner and Korganova, 1995), *Apodera vas* (Zapata and Fernández, 2008), and *Trinema penardi* (Luketa, 2016). In the present study, the *Sphagnum*-dwelling population of *Padaungiella lageniformis* near Alagovac Lake demonstrated a new example of size discontinuous polymorphism.

Wailes (1912) described *Nebela lageniformis* var. *minor* from the North America and noted that a small form, which differed from the type in size only, was not uncommon; the individuals observed varied from 85–100 μ in length. Deflandre (1936) proposed new taxonomic status and name for this taxon: *Nebela wailesi*. Kosakyan et al. (2012) based on molecular data included this species into the genus *Padaungiella*. However, Luketa (2015) described size-monomorphic population of *P. lageniformis* with specimens characterized by shell length ranging between 91 and 127 μm. Also, moss-dwelling population from the Alagovac Lake region (East Herzegovina) analyzed in this study showed similar values of shell length (93–128 μm). This population is also size-monomorphic. Meanwhile, *Sphagnum*-dwelling population from the Alagovac Lake region is size-dimorphic, with two morphometric classes: small-size (91–100 μm) and large-size (101–139 μm). Results of the present study suggest that *P. wailesi* is not a taxon but a size-class in some populations of *P. lageniformis*. For this reason, I suggest synonymizing *P. wailesi* with *P. lageniformis*.

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